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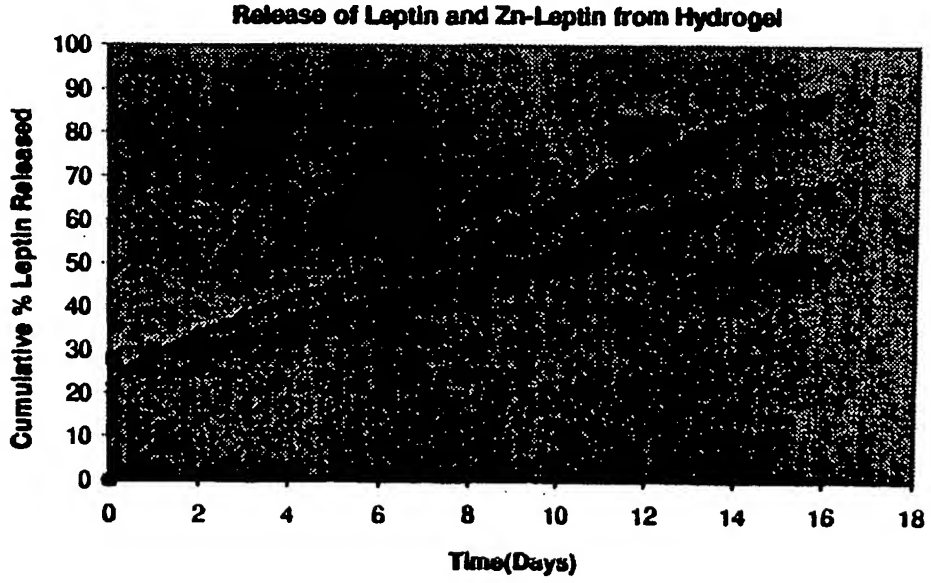

(57) Abstract: The present invention relates to the use of thermosensitive, biodegradable hydrogels, consisting of a block copolymer of poly(d,1- or 1-lactic acid) (PLA) or poly(lactide-co-glycolide) (PLGA) and low molecular weight Pluronics, for the sustained delivery of biologically active agents.

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THERMOSENSITIVE BIODEGRADABLE HYDROGELS BASED ON LOW MOLECULAR WEIGHT PLURONICS

FIELD OF THE INVENTION

The present invention relates to the use of thermosensitive, biodegradable hydrogels, consisting of a block copolymer of poly(d,l- or l-lactic acid)(PLA) or poly(lactide-co-glycolide)(PLGA) and low molecular weight Pluronics, for the sustained delivery of biologically active agents.

BACKGROUND OF THE INVENTION

Due to recent advances in genetic and cell engineering technologies, proteins known to exhibit various pharmacological actions *in vivo* are capable of production in large amounts for pharmaceutical applications. Such proteins include erythropoietin (EPO), granulocyte colony-stimulating factor (G-CSF), interferons (alpha, beta, gamma, consensus), tumor necrosis factor binding protein (TNFbp), interleukin-1 receptor antagonist (IL-1ra), brain-derived neurotrophic factor (BDNF), kerantinocyte growth factor (KGF), stem cell factor (SCF), megakaryocyte growth differentiation factor (MGDF), osteoprotegerin (OPG), glial cell line derived neurotrophic factor (GDNF), novel erythropoiesis stimulating factor (NESP) and obesity protein (OB protein). OB protein may also be referred to herein as leptin.

Because proteins such as leptin generally have short *in vivo* half-lives and negligible oral bioavailability, they are typically administered by frequent injection, thus posing a significant physical burden on the patient (*e.g.*, injection site reactions

are particularly problematic with many leptin formulations) and associated administrative costs. As such, there is currently a great deal of interest in developing and evaluating sustained-release formulations. Effective sustained-release formulations can provide a means of controlling blood levels of the active ingredient, and also provide greater efficacy, safety, patient convenience and patient compliance. Unfortunately, the instability of most proteins (*e.g.* denaturation and loss of bioactivity upon exposure to heat, organic solvents, etc.) has greatly limited the development and evaluation of sustained-release formulations.

Biodegradable polymer matrices have thus been evaluated as sustained-release delivery systems. Attempts to develop sustained-release formulations have included the use of a variety of biodegradable and non-biodegradable polymer (*e.g.* poly(lactide-co-glycolide)) microparticles containing the active ingredient (*see e.g.*, Wise et al., *Contraception*, 8:227-234 (1973) and Hutchinson et al., *Biochem. Soc. Trans.*, 13:520-523 (1985)), and a variety of techniques are known by which active agents, *e.g.* proteins, can be incorporated into polymeric microspheres (*see e.g.*, U.S. Patent No. 4,675,189 and references cited therein).

Utilization of the inherent biodegradability of these materials to control the release of the active agent and provide a more consistent sustained level of medication provides improvements in the sustained release of active agents. Unfortunately, some of the sustained release devices utilizing microparticles still suffer from such things as: active agent aggregation formation; high initial bursts of active agent with minimal release thereafter; and incomplete release of active agent.

Other drug-loaded polymeric devices have also been investigated for long term, therapeutic treatment of various diseases, again with much attention being directed to polymers derived from alpha hydroxycarboxylic acids, especially lactic acid in both its racemic and optically active form, and glycolic acid, and copolymers thereof. These polymers are commercially available and have been utilized in FDA-approved systems, *e.g.*, the Lupron Depot™, which consists of injectable microcapsules which release leuprolide acetate for about 30 days for the treatment of prostate cancer.

Various problems identified with the use of such polymers include: inability of certain macromolecules to diffuse out through the matrix; deterioration and decomposition of the drug (*e.g.*, denaturation caused by the use of organic solvents); irritation to the organism (*e.g.* side effects due to use of organic solvents); low biodegradability (such as that which occurs with polycondensation of a polymer with a multifunctional alcohol or multifunctional carboxylic acid, *i.e.*, ointments); and slow rates of polymer degradation.

The use of polymers which exhibit reverse thermal gelation have also been reported. For example, Okada et al., Japanese Patent Application 2-78629 (1990) describe biodegradable block copolymers synthesized by transesterification of poly(lactic acid)(PLA) or poly(lactic acid)/glycolic acid (PLA/GA) and poly(ethylene glycol)(PEG). PEGs with molecular weights ranging from 200 to 2000, and PLA/GA with molecular weights ranging from 400 to 5000 were utilized. The resultant product was miscible with water and formed a hydrogel. The Okada et al.

reference fails to provide any demonstration of sustained delivery of drugs using the hydrogels.

Cha et al., U.S. Patent No. 5,702,717 (Dec. 30, 1997) describe systems for parenteral delivery of a drug comprising an injectable biodegradable block copolymeric drug delivery liquid having reverse thermal gelation properties, i.e., ability to form semi-solid gel, emulsions or suspension at certain temperatures. Specifically, these thermosensitive gels exist as a mobile viscous liquid at low temperatures, but form a rigid semisolid gel at higher temperatures. Thus, it is possible to use these polymers to design a formulation which is liquid at room temperature or at lower temperature and below, but gels once injected, thus producing a depot of drug at the injection site. The systems described by Cha et al. utilize a hydrophobic A polymer block comprising a member selected from the group consisting of poly(α-hydroxy acids) and poly(ethylene carbonates) and a hydrophilic B polymer block comprising a PEG. The Cha et al. system requires that less than 50% by weight hydrophobic A polymer block be utilized and greater than 50% by weight hydrophilic B polymer block be utilized. Interestingly, however, it appears that several of the disclosed hydrogels might not be commercially useful in that the lower critical solution temperature (LCST) for many of the gels is greater than 37°C. Although Cha et al. propose use of their hydrogels for controlled release of drugs, no such demonstration is provided.

Martini et al., *J. Chem. Soc.*, 90(13):1961-1966 (1994) describe low molecular weight ABA type triblock copolymers which utilize hydrophobic poly(ε-caprolactone)(PCL) and PEG. Unfortunately, *in*

vitro degradation rates for these copolymers was very slow, thus calling into question their ability as sustained-release systems.

Pluronics (a tri-block copolymer of poly(ethylene oxide) and poly(propylene oxide)) is a commercially available polymer which exhibits thermoreversible gelation properties in aqueous medium. As such, Pluronics have also been identified as a suitable vehicle for parenteral sustained delivery applications; *see, e.g.*, Stratton et al., PCT/US97/13479 (WO 98/02142) January 22, 1998; Wang et al., *Inter. Jour. Pharmaceutics*, 113:73-81 (1995).

Unfortunately, current systems utilizing Pluronics suffer from the fact that they are toxic to body organs and are nonbiodegradable. Moreover, only high molecular weight Pluronics such as F-127 (MW 12,600 daltons) at higher concentrations (25-40 wt.%) exhibit thermoreversible gelation. Finally, most studies utilizing Pluronics demonstrated a sustained release of drugs or proteins for a very short duration.

There still exists a need for thermosensitive, biodegradable hydrogels which provide for instant gelation, and which possess the necessary rate of degradation to make use of the hydrogels to provide for sustained-release of the biologically active agent commercially practical.

SUMMARY OF THE INVENTION

It is thus an object of the present invention to provide pharmaceutical compositions comprising an effective amount of a biologically active agent incorporated into a polymeric matrix, said polymeric matrix comprising a block copolymer which is biodegradable, exhibits thermal gelation behavior, and

is capable of providing for the sustained-release of the biologically active agent.

A further object of the present invention is to provide a method for the parenteral administration of a biologically active agent in a biodegradable polymeric matrix to a warm blooded animal, wherein a gel depot is formed within the body of said animal and the biologically active agent is released from the depot at a controlled rate concomitant with biodegradation of the polymeric matrix.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts the two methods by which the A-B-A block copolymers of the present invention can be prepared.

Figure 2 depicts the *in vitro* release characteristics of leptin (♦) and Zn-leptin (■) released from a hydrogel (PLGA/Pluronics (60%/40% w/w)). % protein released is plotted vs. time (days).

Figure 3 depicts the *in vivo* bioactivity for various leptin-containing hydrogel (PLGA/Pluronics (60%/40% w/w)) formulations. The -□- depicts a 20mM acetate, pH 4.8, buffer control, 100µl on day 0; -•- depicts a hydrogel (60%/40%) control, 100µl on day 0; -▲- depicts leptin (20 mg/mL), 100 mg/kg, 100µl on day 0; and -■- depicts a leptin-containing hydrogel (PLGA/Pluronics (60%/40% w/w)), 20 mg/mL leptin, 100 mg/kg, 100µl on day 0. % body weight change (from the day 0 body weight) is plotted vs. time (days).

DETAILED DESCRIPTION OF THE INVENTION

As used herein, the following terms shall have the following meaning:

"Reverse thermal gelation" is defined as meaning the temperature below which a copolymer is soluble in water and above which the block copolymer forms a semi-solid, i.e. gels, emulsions, dispersions and suspensions.

"LCST", or lower critical solution temperature, is defined as meaning the temperature at which a biodegradable block copolymer undergoes reverse thermal gelation. For purposes of the present invention, the term "LSCT" can be used interchangeably with "reverse thermal gelation temperature".

"Depot" is defined as meaning a drug delivery liquid which, following injection into a warm blooded animal, has formed a gel upon having the temperature raised to or above the LCST.

"Biodegradable" is defined as meaning that the block copolymer will erode or degrade *in vivo* to form smaller non-toxic components.

"Parenteral administration" is defined as meaning any route of administration other than the alimentary canal, including, for example, subcutaneous and intramuscular.

The present invention involves utilization of block copolymers having hydrophobic ("A") block segments and hydrophilic ("B") block segments. The block copolymers are triblock copolymers, *e.g.*, ABA or BAB type block copolymers, which possess reverse thermal gelation properties and are biodegradable and biocompatible. Alternatively, the block copolymers are diblock copolymers, *e.g.*, AB or BA. Importantly, triblock copolymers of the present invention provide

instant gelation and possess the necessary rate of degradation to be commercially useful.

Biodegradable hydrophobic A block segments contemplated for use include poly(α-hydroxy acid) members derived from or selected from the group consisting of homopolymers and copolymers of poly(lactide)s (d,l- or l- forms), poly(glycolide)s, polyanhydrides, polyorthoesters, polyetheresters, polycaprolactone, polyesteramides, polycarbonate, polycyanoacrylate, polyurethanes, polyacrylate, blends and copolymers thereof.

The term "PLGA" as used herein is intended to refer to a polymer of lactic acid alone, a polymer of glycolic acid alone, a mixture of such polymers, a copolymer of glycolic acid and lactic acid, a mixture of such copolymers, or a mixture of such polymers and copolymers. Preferably, the biodegradable A block polymer will be poly lactide-co-glycolide (PLGA), and the PLGA composition will be such that the necessary rate of gelation and rate of degradation are obtained.

The range of molecular weights contemplated for the polymers to be used in the present processes can be readily determined by a person skilled in the art based upon such factors the desired polymer degradation rate. Typically, the range of molecular weight for the A block will be 1000 to 20,000 Daltons.

Hydrophilic B block segments contemplated for use include Pluronics having average molecular weights of between about 1000 and 6000.

The copolymer compositions for the block copolymers of the present invention are specially regulated to assure retention of the desired water-solubility and gelling properties, i.e., the ratios must be such that the block copolymers possess water solubility at temperatures below the LCST, and such

that there is instant gelation under physiological conditions (i.e. pH 7.0 and 37°C) so as to minimize the initial burst of drug. In the hydrogels of the present invention the hydrophobic A block makes up 20% to 80% by weight of the copolymer and the hydrophilic B block makes up 20% to 80% of the copolymer.

The concentration at which the block copolymers of the present invention remain soluble below the LCST are generally up to about 60% by weight, with 10%-30% preferred. The concentration utilized will depend upon the copolymer composition actually used, as well as whether or not a gel or emulsion is desired.

The thermosensitive block copolymers of the present invention can be prepared by thermal condensation. In a typical experiment, A-B-A block copolymers of PLGA/PLA (block A) and Pluronics (block B) are synthesized by mixing either homopolymer of poly lactide (PLA) or copolymer of poly lactide-co-gycolide (PLGA) with Pluronics and allowing di-hydroxy Pluronics to react with PLGA or PLA at 160°C under reduced pressure. Different weight ratios of PLGA and Pluronics were used for thermal condensation to obtain a series of block copolymers with desirable copolymer composition and block lengths. Copolymer composition and relative block lengths were confirmed by ^{1}H-NMR spectroscopy.

Alternatively, the copolymers could be synthesized in a melt process which involves ring opening polymerization of A block using B block as the initiator. In a typical experiment, the ABA triblock copolymer is prepared by stannous octoate catalyzed ring-opening polymerization of d,l-dilactide (or PLGA) using α,ω-dihydroxy-terminated Pluronics as the

initiator. The mole ratio of B block to d,l-dilactide (or PLGA) is used to control the lengths of the A blocks, and provide a series of polymers with increasing A block contents and hydrophobicities. The relative A and B block lengths can be confirmed by ^{1}H-NMR spectroscopy.

The process used to mix the copolymers with a biologically active agent and/or other materials involves dissolving the ABA block copolymers in an aqueous solution, followed by addition of the biologically active agent (in solution, suspension or powder), followed by thorough mixing to assure a homogeneous mixing of the biologically active agent throughout the copolymer. Alternatively, the process can involve the dissolving of the ABA block copolymer in a biologically active agent-containing solution. In either case, the process is conducted at a temperature lower than the gelation temperature of the copolymer and the material is implanted into the body as a solution which then gels or solidifies into a depot in the body. In the compositions of the present invention, the biologically active agent will generally have a concentration in the range of 0 to 200 mg/mL.

Buffers contemplated for use in the preparation of the biologically active agent-containing hydrogels are buffers which are all well known by those of ordinary skill in the art and include sodium acetate, Tris, sodium phosphate, MOPS, PIPES, MES and potassium phosphate, in the range of 25mM to 500mM and in the pH range of 4.0 to 8.5.

It is also envisioned that other excipients, e.g., various sugars, salts, or surfactants, may be included in the biologically active agent-containing hydrogels of the present invention in order to alter the LCST or rate of gelation of the gels. The ability

to alter the rate of gelation and/or LCST is important and an otherwise non-useful hydrogel may be made useful by addition of such excipients. Examples of such sugars include glucose or sucrose in the range of 5% to 20%. Examples of such salts include sodium chloride or zinc chloride in the range of 0.5% to 10%.

As used herein, biologically active agents refers to recombinant or naturally occurring proteins, whether human or animal, useful for prophylactic, therapeutic or diagnostic application. The biologically active agent can be natural, synthetic, semi-synthetic or derivatives thereof. In addition, biologically active agents of the present invention can be perceptible. A wide range of biologically active agents are contemplated. These include but are not limited to hormones, cytokines, hematopoietic factors, growth factors, antiobesity factors, trophic factors, anti-inflammatory factors, small molecules and enzymes (see also U.S. Patent No. 4,695,463 for additional examples of useful biologically active agents). One skilled in the art will readily be able to adapt a desired biologically active agent to the compositions of present invention.

Proteins contemplated for use would include but are not limited to interferon consensus (*see*, U.S. Patent Nos. 5,980,884, 5,372,808, 5,541,293 4,897,471, 5,661,009 and 4,695,623 hereby incorporated by reference including drawings), interleukins (*see*, U.S. Patent No. 5,075,222, hereby incorporated by reference including drawings), erythropoietins (*see*, U.S. Patent Nos. 4,703,008, 5,441,868, 5,618,698 5,547,933, and 5,621,080 hereby incorporated by reference including drawings), granulocyte-colony stimulating factors (*see*, U.S. Patent Nos. 4,810,643, 4,999,291, 5,581,476, 5,582,823, and PCT Publication No. 94/17185, hereby

incorporated by reference including drawings), stem cell factor (PCT Publication Nos. 91/05795, 92/17505 and 95/17206, hereby incorporated by reference including drawings), novel erythropoiesis stimulating protein (NESP) (PCT Publication No. 94/09257, hereby incorporated by reference including drawings) and leptin (OB protein) (*see* PCT publication Nos. 96/40912, 96/05309, 97/00128, 97/01010 and 97/06816 hereby incorporated by reference including figures).

Also contemplated for incorporation into the compositions of the present invention are derivatives, fusion proteins, conjugates, analogs or modified forms of the natural active ingredients. Chemical modification of biologically active proteins has been found to provide additional advantages under certain circumstances, such as increasing the stability and circulation time of the therapeutic protein and decreasing immunogenicity. For example, U.S. Patent No. 4,179,337, Davis et al., issued December 18, 1979, discloses conjugation of water-soluble polypeptides such as enzymes and insulin to polyethylene glycol (PEG); *see also* WO 87/00056, published January 15, 1987.

Another type of chemical modification contemplated for the active ingredients of the present invention is succinylation. The properties of various succinylated proteins are described in Holcenberg et al., *J. Biol. Chem,* 250:4165-4170 (1975), and WO 88/01511 (and references cited therein), published March 10, 1988.

The present leptins used are preferably those with amino acid sequence of natural human OB protein; see Zhang et al., *Nature,* 372:425-432 (1994); *see also,* the Correction at *Nature,* 374:479 (1995), optionally with an N-terminal methionyl residue incident to

bacterial expression is used. (*See*, Materials and Methods, *infra*). PCT publication No. WO 96/05309, published February 22, 1996, entitled, "Modulators of Body Weight, Corresponding Nucleic Acids and Proteins, and Diagnostic and Therapeutic Uses Thereof" fully sets forth OB protein and related compositions and methods, and is herein incorporated by reference. An amino acid sequence for human OB protein is set forth at WO 96/05309 SEQ. ID No:4 and 6 (at pages 172 and 174 of that publication), and the first amino acid residue of the mature protein is at position 22 and is a valine residue. The mature protein is 146 residues (or 145 if the glutamine at position 49 is absent, SEQ. ID No:4).

Specific leptin derivatives contemplated for use in the present invention include Fc-leptin fusions, succinylated-leptin, and zinc derivatized leptin. It is desirable to have such leptin containing sustained-release compositions as such compositions could serve to enhance the effectiveness of either exogenously administered or endogenous leptin, or could be used, for example, to reduce or eliminate the need for exogenous leptin administration. Moreover, because the materials utilized in the present invention are biocompatible and biodegradable, use of the protein compositions of the present invention help prevent adverse injection site reactions sometimes associated with *i.v.* injections of various proteins such as leptin.

In addition, biologically active agents can also include insulin, gastrin, prolactin, adrenocorticotropic hormone (ACTH), thyroid stimulating hormone (TSH), luteinizing hormone-releasing hormone (LHRH), follicle stimulating hormone (FSH), human chorionic gonadotropin (HCG), motilin, interferons (alpha, beta, gamma), tumor necrosis factor (TNF),

tumor necrosis factor-binding protein (TNF-bp), interleukin-1 receptor antagonist (IL-1ra), brain derived neurotrophic factor (BDNF), glial derived neurotrophic factor (GDNF), neurotrophic factor 3 (NT3), fibroblast growth factors (FGF), neurotrophic growth factor (NGF), bone growth factors such as osteoprotegerin (OPG), insulin-like growth factors (IGFs), macrophage colony stimulating factor (M-CSF), granulocyte macrophage colony stimulating factor (GM-CSF), megakaryocyte derived growth factor (MGDF), keratinocyte growth factor (KGF), thrombopoietin, platelet-derived growth factor (PGDF), colony simulating growth factors (CSFs), bone morphogenetic protein (BMP), superoxide dismutase (SOD), tissue plasminogen activator (TPA), urokinase, streptokinase and kallikrein. The term proteins, as used herein, includes peptides, polypeptides, consensus molecules, analogs, derivatives or combinations thereof.

Also included are those polypeptides with amino acid substitutions which are "conservative" according to acidity, charge, hydrophobicity, polarity, size or any other characteristic known to those skilled in the art. *See generally*, Creighton, Proteins, W.H. Freeman and Company, N.Y., (1984) 498 pp. plus index, *passim*. One may make changes in selected amino acids so long as such changes preserve the overall folding or activity of the protein. Small amino terminal extensions, such as an amino-terminal methionine residue, a small linker peptide of up to about 20-25 residues, or a small extension that facilitates purification, such as a poly-histidine tract, an antigenic epitope or a binding domain, may also be present. *See*, in general, Ford et al., *Protein Expression and Purification*, 2:95-107 (1991), which is

herein incorporated by reference. Polypeptides or analogs thereof may also contain one or more amino acid analogs, such as peptidomimetics.

In general, comprehended by the invention are pharmaceutical compositions comprising effective amounts of chemically modified protein, or derivative products, together with pharmaceutically acceptable diluents, preservatives, solubilizers, emulsifiers, adjuvants and/or carriers needed for administration. (See PCT 97/01331 hereby incorporated by reference.) The optimal pharmaceutical formulation for a desired biologically active agent will be determined by one skilled in the art depending upon the route of administration and desired dosage. Exemplary pharmaceutical compositions are disclosed in Remington's Pharmaceutical Sciences (Mack Publishing Co., 18th Ed., Easton, PA, pgs. 1435-1712 (1990)).

The pharmaceutical compositions of the present invention are administered as a liquid via intramuscular or subcutaneous route and undergo a phase change wherein a gel is formed within the body, since the body temperature will be above the gelation temperature of the material. The release rates and duration for the particular biologically active agents will be a function of, *inter alia*, hydrogel density and the molecular weight of the agent.

Therapeutic uses of the compositions of the present invention depend on the biologically active agent used. One skilled in the art will readily be able to adapt a desired biologically active agent to the present invention for its intended therapeutic uses. Therapeutic uses for such agents are set forth in greater detail in the following publications hereby incorporated by reference including drawings. Therapeutic uses include but are not limited to uses

for proteins like interferons (*see*, U.S. Patent Nos. 5, 980,884, 5,372,808, hereby incorporated by reference including drawings), interleukins (*see*, U.S. Patent No. 5,075,222, hereby incorporated by reference including drawings), erythropoietins (*see*, U.S. Patent Nos. 4,703,008, 5,441,868, 5,618,698 5,547,933, and 5,621,080 hereby incorporated by reference including drawings), granulocyte-colony stimulating factors (*see*, U.S. Patent Nos. 4,999,291, 5,581,476, 5,582,823, 4,810,643 and PCT Publication No. 94/17185, hereby incorporated by reference including drawings), stem cell factor (PCT Publication Nos. 91/05795, 92/17505 and 95/17206, hereby incorporated by reference including drawings), novel erythropoiesis stimulating protein (NESP) (PCT Publication No. 94/09257, hereby incorporated by reference including drawings) and the OB protein (*see* PCT publication Nos. 96/40912, 96/05309, 97/00128, 97/01010 and 97/06816 hereby incorporated by reference including figures). In addition, the present compositions may also be used for manufacture of one or more medicaments for treatment or amelioration of the conditions the biologically active agent is intended to treat.

In the sustained-release compositions of the present invention, an effective amount of active ingredient will be utilized. As used herein, sustained release refers to the gradual release of active ingredient from the polymer matrix, over an extended period of time. The sustained release can be continuous or discontinuous, linear or non-linear, and this can be accomplished using one or more polymer compositions, drug loadings, selection of excipients, or other modifications. The sustained release will result in biologically effective serum levels of the active agent (typically above endogenous levels) for a

period of time longer than that observed with direct administration of the active agent. Typically, a sustained release of the active agent will be for a period of a week or more, preferably up to one month.

The following examples are offered to more fully illustrate the invention, but are not to be construed as limiting the scope thereof.

Materials

PLGA (poly Lactic acid-co-Glycolic acid) and PLA (poly Lactic acid) were synthesized by direct thermal condensation of glycolic acid and lactic acid at 180°C under reduced pressure. Pluronics® were obtained from BASF. Leptin and GCSF were obtained from Amgen Inc. All other chemicals are from sources well known in the art.

Example 1

This example describes synthesis of a PLGA/Pluronics, A-B-A (PLGA-Pluronics-PLGA), block copolymer by thermal condensation. The thermal condensation method is generally depicted in Figure 1, Scheme 2.

30 g PLGA (75/25 LA/GA ratio) (Mn 3740, MW 7050 by gel permeation chromatography (GPC)) and 20.0 g Pluronics® L35 (MW 1900) were placed into a three-neck round bottom flask equipped with a thermometer, a nitrogen gas inlet, and a distillation condenser connected to a vacuum pump. After addition of the polymers, the temperature of the reaction mixture was raised slowly to 160°C under nitrogen purging. The condensation reaction was further carried out at 160°C for 24-30 hours under 500 millitorr pressure and with

continuous bubbling of dry nitrogen gas. At the end of the condensation reaction, the reaction mixture was cooled, dissolved in methylene chloride and precipitated with an excess of cold isopropanol, followed by ether wash.

The isolated polymer was dried at 30°C under vacuum for 48 hours. The molecular weight of the block copolymer was determined by gel permeation chromatography (GPC) using polystyrene standards. The copolymer composition and relative block lengths were determined by ^{1}H-NMR.

The PLGA/Pluronics block copolymer dissolved either in 100mM sodium acetate, pH 6.0, or 100mM sodium phosphate, pH 7.0, exhibited a unique thermoreversible property (solution below room temperature and gel above room temperature, sol-gel-sol) with lower critical solution temperature (LCST) at about 30°C.

Example 2

This example describes the synthesis of PLGA/Pluronics, A-B-A (PLGA-Pluronics-PLGA) block copolymers by ring opening polymerization. The ring opening polymerization method is generally depicted in Figure 1, Scheme 1.

DL-Lactide 6.0 g, 0.5% Stanneous 2-ethyl hexanoate and 4.0 g Pluronics® L35 (MW 1900) were placed into a three-neck round bottom flask equipped with a nitrogen gas inlet. The polymerization was carried out at 140°C for 20-24 hrs. At the end of reaction, the reaction mixture was cooled, dissolved in methylene chloride and precipitated with an excess of cold isopropanol followed by ether wash. The isolated polymer was dried at 30°C under vacuum for 48 hours.

The molecular weight of the block copolymer was determined by GPC using polystyrene standards. The copolymer composition and relative block lengths were determined by ^{1}H-NMR.

The block copolymer dissolved either in 100mM sodium acetate, pH 6.0, or 100mM sodium phosphate, pH 7.0, exhibited a unique thermoreversible (sol-gel-sol) gelation.

Example 3

This example describes the synthesis of PLGA/Pluronics, A-B-A (PLGA-Pluronics-PLGA), block copolymers using different weight ratios of PLGA and Pluronics® L35 (MW 1900).

The synthesis and characterization procedure described in Example 1 were utilized to prepare PLGA/Pluronics block copolymers with various PLGA to Pluronics® L35 ratios (see Table 1 below). All of the block copolymers listed below showed thermoreversible (sol-gel-sol) gelation.

Table 1

PLGA (LA/GA ratio) wt (g)	Pluronics®L35 wt (g)	PLGA/Pluronics wt ratio	PLGA/Pluronics ratio by NMR
PLGA (75/25%) 30g (Mn 1922, MW 2920)	12.86g	70/30	1.70
PLGA (75/25%) 30g (Mn 3500, MW 7020)	15.45g	66/34	1.44
PLGA (75/25%) 30g (Mn 3500, MW 7020)	16.15g	65/35	1.38
PLGA (75/25%) 30g (Mn 3500, MW 7020)	20.00g	60/40	1.07
PLGA (75/25%) 30g (Mn 3500, MW 7020)	21.72g	58/42	1.03
PLGA (75/25%) 30g (Mn 3500, MW 7020)	25.55g	54/46	0.87
PLGA (75/25%) 30g (Mn 3500, MW 7020)	30.00g	50/50	0.74

Example 4

This example describes the synthesis of PLGA/Pluronics, A-B-A (PLGA-Pluronics-PLGA), block copolymers using different Pluronics.

The synthesis and characterization procedure described in Example 1 were utilized to prepare PLGA/Pluronics block copolymers with PLGA/Pluronics wt. ratio = 60/40 (See Table 2). All of the block copolymers listed below showed thermoreversible (sol-gel-sol) gelation.

Table 2

PLGA (LA/GA ratio)	Pluronics® Type	PLGA/Pluronics wt ratio	PLGA/Pluronics ratio by NMR
PLGA (75/25%) (Mn 3500, MW 7020)	L35 (Mw 1900)	60/40	1.07
PLGA (75/25%) (Mn 3500, MW 7020)	P65 (Mw 3400)	60/40	1.26
PLGA (75/25%) (Mn 3500, MW 7020)	L44 (Mw 2200)	60/40	1.15
PLGA (75/25%) (Mn 3500, MW 7020)	L43 (Mw 1850)	60/40	1.13

Example 5

This example describes the synthesis of PLGA/Pluronics® L35, A-B-A (PLGA-Pluronics-PLGA), block copolymers using PLGA with different lactic acid to glycolic acid ratios.

The synthesis and characterization procedures described in Example 1 were utilized to prepare PLGA/Pluronics block copolymers using PLGA with different LA to GA ratios (see Table 3). The block copolymers listed below showed thermoreversibile (sol-gel-sol) gelation.

Table 3

PLGA (LA/GA ratio) wt (g)	Pluronics®L35 wt (g)	PLGA/Pluronics wt ratio	PLGA/Pluronics ratio by NMR
PLGA (100/0%) 30g	20.00g	65/35	2.68
PLGA (75/25%) 30g	16.15g	65/35	1.38
PLGA (50/50%) 30g	14.77g	67/33	1.59
PLGA (56/44%) 40g	21.53g	65/35	1.40

Example 6

This example describes the preparation of a leptin/hydrogel formulation and the methods used to determine the *in vitro* release kinetics and *in vivo* pharmacodynamics effect.

Preparation of leptin/hydrogel formulation

The PLGA/Pluronics block copolymer described in Example 1 (PLGA/Pluronics®L35 wt. ratio = 60/40) was dissolved in 50mM sodium acetate, pH 6.0. Leptin solution (formulated in 20mM acetate, pH 4.8) was slowly added to the hydrogel solution and the mixture was gently swirled on an orbital shaker at 5°C to assure a homogeneous mixing of leptin throughout the hydrogel solution. The final concentration of the block copolymer in the final leptin/hydrogel formulation was in the range of 10-50% (w/w) and the leptin concentration was in the range of 0-100 mg/ml. The final leptin/hydrogel formulation was filtered through 0.2μ filter and stored either as a solution at 5°C or stored as a frozen mass at -20°C.

Alternatively, the leptin/hydrogel formulation can be prepared by dissolving the

PLGA/Pluronics block copolymer in a leptin solution. The leptin solution concentration was varied to obtain desirable copolymer as well as the desired protein concentration in final formulation.

***In vitro* Release Study**

The *in vitro* release of leptin from the leptin/hydrogel was carried out in 20mM sodium phosphate, 5% sorbitol, pH 7.4, at 37°C. 1 ml of leptin/hydrogel solution formulation was placed in a glass vial at 37°C. Upon gelation of the leptin/hydrogel formulation, 1 ml of 20mM phosphate, 5% sorbitol, pH 7.4, buffer was added directly above and in contact with the gel. The amount of leptin released in the top buffer phase was determined by UV spectrophotometer at 280nm as well as by SEC-HPLC at 220nm. To maintain a perfect sink condition the aqueous receptor phase above the gel was completely removed at definite time intervals and replaced by fresh buffer. The % leptin released over time is depicted in Figure 2. The integrity of the leptin released from the hydrogel formulation was confirmed by HPLC and gel electrophoresis (SDS-PAGE).

***In vivo* bioactivity study**

The *in vivo* bioactivity of leptin/hydrogel formulations was evaluated in normal mice. Mice were injected subcutaneously (s.c.) with either: a) 0.1 ml of 20mM acetate buffer, pH 4.8, (n=5, day 0 only); (b) 0.1 ml of 20 mg/ml leptin formulated in 20mM acetate buffer, pH 4.8 (n=5, 100 mg/kg, day 0 only); (c) 0.1 ml of a leptin/hydrogel formulation consisting of 20 mg/ml leptin, in 20mM acetate, pH 4.8 (n=5, 100

mg/kg, day 0 only); or (d) 0.1 ml of a hydrogel control, formulated in 50mM acetate, pH 6.0 (n=5, day 0 only).

% body weight change (from the day 0 body weight) was determined by weighing the animals daily until the body weight of the animals injected with sample (b),(c) reached the body weights of the animals injected with buffer control (sample (a)). Importantly, a single *s.c.* injection of 100 mg/kg leptin/hydrogel formulation (sample (c)) showed sustained weight loss in normal mice over a 5 day period (See Figure 3).

Example 7

This example describes incorporation of a Zn:leptin suspension into PLGA/Pluronics hydrogel and the results of *in vivo* release kinetics of the leptin from the Zn:leptin/hydrogel.

The PLGA/Pluronics block polymers described in the Example 1 was hydrated in 100mM Tris, pH 8.0 buffer. The final pH of the hydrogel solution was maintained between 6.5 - 7.0 and then a zinc chloride solution was added to the hydrogel to obtain a 0.1mM $ZnCl_2$ concentration in the final hydrogel solution. To this hydrogel solution, a Zn:leptin suspension was added as described in Example 6. The final Zn:leptin concentration in the hydrogel described in this example was 20 mg/ml. The *in vitro* release and *in vivo* bioactivity of a Zn:leptin/hydrogel formulation was carried out as described in Example 6. (See Figures 2 and 3).

The present invention has been described in terms of particular embodiments found or proposed to comprise preferred modes for the practice of the invention. It will be appreciated by those of ordinary skill in the art that, in light of the present disclosure, numerous modifications and changes can be made in the particular embodiments exemplified without departing from the intended scope of the invention.

WHAT IS CLAIMED IS:

1. A pharmaceutical composition for the sustained administration of an effective amount of a biologically active agent, or a derivative, analog, fusion, conjugate, or chemically modified form thereof, comprising an injectable biodegradable polymeric matrix into which said biologically active agent has been incorporated, said polymeric matrix having reverse thermal gelation properties, and wherein said injectable polymeric matrix is maintained at a temperature below the lower critical solution temperature of said polymeric matrix.

2. The composition of claim 1, wherein said polymeric matrix is a biodegradable block copolymer comprising:

(a) 20% to 80% by weight of a hydrophobic A polymer block and;

(b) 20% to 85% by weight of a hydrophilic B polymer block comprising a Pluronics having an average molecular weight of between 1000-6000.

3. The composition of claim 2, wherein said hydrophobic A polymer block is a poly(α-hydroxy acid) having an average molecular weight of between 1000-20,000.

4. The composition of claim 3, wherein said poly(α-hydroxy acid) is selected from the group consisting of poly(lactide)s (d,l- or l- forms), poly(glycolide)s, polyanhydrides, polyorthoesters, polyetheresters, polycaprolactone, polyesteramides,

polycarbonate, polycyanoacrylate, polyurethanes, polyacrylate, blends and copolymers thereof.

5. The composition of claim 4, wherein said poly(α-hydroxy acid) is poly lactide-co-glycolide (PLGA).

6. The composition of claim 5, wherein said block copolymer is a triblock copolymer having a configuration selected from the group consisting of ABA or BAB block segments.

7. The composition of claim 6, wherein said hydrophobic A polymer block comprises 60% by weight of said block copolymer and said hydrophilic B polymer block comprises 40% by weight of said block copolymer.

8. The composition of claim 7 further comprising an excipient which will vary the lower critical solution temperature and increase the rate of gelation of said block copolymer.

9. The composition of claim 1, wherein said biologically active agent is a protein selected from the group consisting of interferon consensus, interleukins, erythropoietins, granulocyte-colony stimulating factor (GCSF), stem cell factor (SCF), leptin (OB protein), interferons (alpha, beta, gamma), tumor necrosis factor (TNF), tumor necrosis factor-binding protein (TNF-bp), interleukin-1 receptor antagonist (IL-1ra), brain derived neurotrophic factor (BDNF), glial derived neurotrophic factor (GDNF), neurotrophic factor 3 (NT3), fibroblast growth factors (FGF), neurotrophic growth factor (NGF), bone growth factors such as osteoprotegerin (OPG), granulocyte

macrophage colony stimulating factor (GM-CSF), megakaryocyte derived growth factor (MGDF), keratinocyte growth factor (KGF), thrombopoietin, platelet-derived growth factor (PGDF), tissue plasminogen activator (TPA), novel erythropoiesis stimulating protein (NESP), urokinase, streptokinase and kallikrein.

10. The composition of claim 1, wherein said biologically active agent is a small molecule.

11. The composition of claim 10, wherein said small molecule is a LHRH antagonist peptide.

12. A method for the parenteral administration of a biologically active agent, or a derivative, analog, fusion, conjugate, or chemically modified form thereof, in a biodegradable polymeric matrix to a warm blooded animal with the resultant sustained release of said agent concomitant with biodegradation of said polymeric matrix, which comprises:

(a) providing an injectable liquid polymeric matrix comprising a biodegradable block copolymer having reverse thermal gelation properties, and into which a biologically active agent has been incorporated;

(b) maintaining said liquid polymeric matrix at a temperature below the lower critical solution temperature of said polymeric matrix; and

(c) injecting said liquid parenterally into said animal, thus forming a gel depot of said drug and polymeric matrix as the temperature of said liquid is raised in the body of said animal above the lower critical solution temperature of the polymeric matrix.

13. The method of claim 12, wherein said polymeric matrix is a biodegradable block copolymer comprising:

(a) 20% to 80% by weight of a hydrophobic A polymer block and;

(b) 20% to 80% by weight of a hydrophilic B polymer block comprising a Pluronics having an average molecular weight of between 1000-6000.

14. The method of claim 13, wherein said hydrophobic A polymer block is a poly(α-hydroxy acid) having an average molecular weight of between 1000-20,000.

15. The method of claim 14, wherein said poly(α-hydroxy acid) is poly lactide-co-glycolide (PLGA).

16. The method of claim 15, wherein said block copolymer is a triblock copolymer having a configuration selected from the group consisting of ABA or BAB block segments.

17. The method of claim 16, wherein said hydrophobic A polymer block comprises 60% by weight of said block copolymer and said hydrophilic B polymer block comprises 40% by weight of said block copolymer.

18. The method of claim 17 further comprising an excipient which will vary the lower critical solution temperature and increase the rate of gelation of said block copolymer.

19. The method of claim 12, wherein said biologically active agent is a protein selected from the group consisting of interferon consensus, interleukins, erythropoietins, granulocyte-colony stimulating factor (GCSF), stem cell factor (SCF), leptin (OB protein), interferons (alpha, beta, gamma), tumor necrosis factor (TNF), tumor necrosis factor-binding protein (TNF-bp), interleukin-1 receptor antagonist (IL-1ra), brain derived neurotrophic factor (BDNF), glial derived neurotrophic factor (GDNF), neurotrophic factor 3 (NT3), fibroblast growth factors (FGF), neurotrophic growth factor (NGF), bone growth factors such as osteoprotegerin (OPG), granulocyte macrophage colony stimulating factor (GM-CSF), megakaryocyte derived growth factor (MGDF), keratinocyte growth factor (KGF), thrombopoietin, platelet-derived growth factor (PGDF), tissue plasminogen activator (TPA), novel erythropoiesis stimulating protein (NESP), urokinase, streptokinase and kallikrein.

20. The method of claim 12, wherein said biologically active agent is a small molecule.

21. The method of claim 20, wherein said small molecule is a LHRH antagonist peptide.

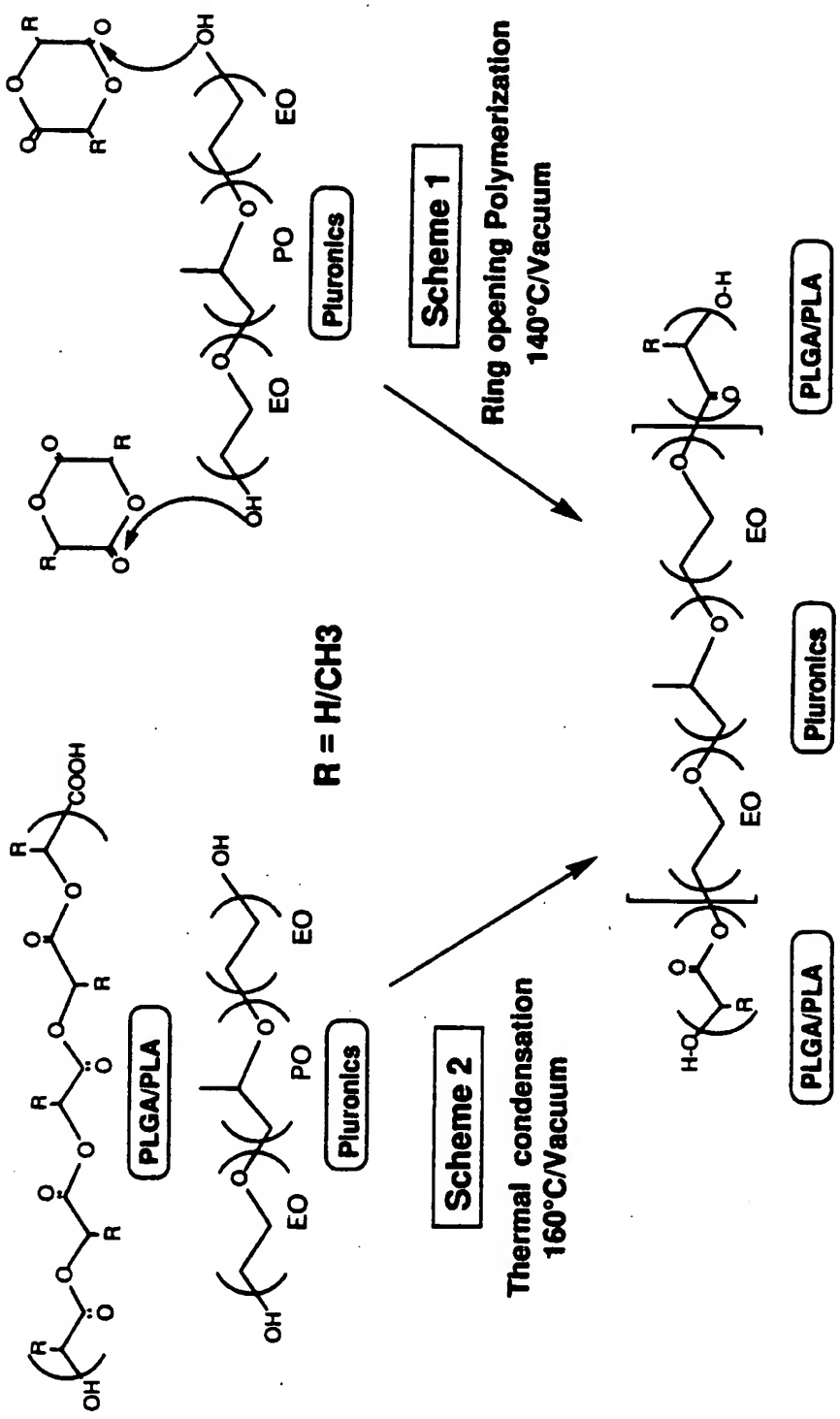
Scheme 1
Ring opening Polymerization
140°C/Vacuum
Scheme 2
Thermal condensation
160°C/Vacuum
R = H/CH3
PLGA/PLA
Pluronics
EO
PO
(A-B-A) BLOCK COPOLYMER

FIGURE 1

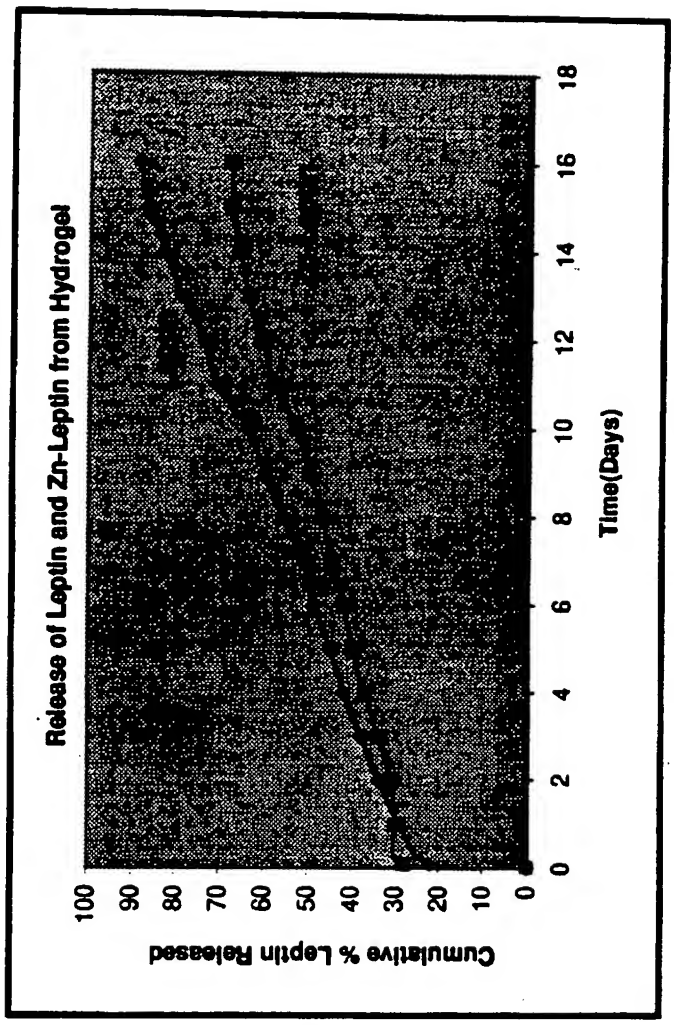

FIGURE 2

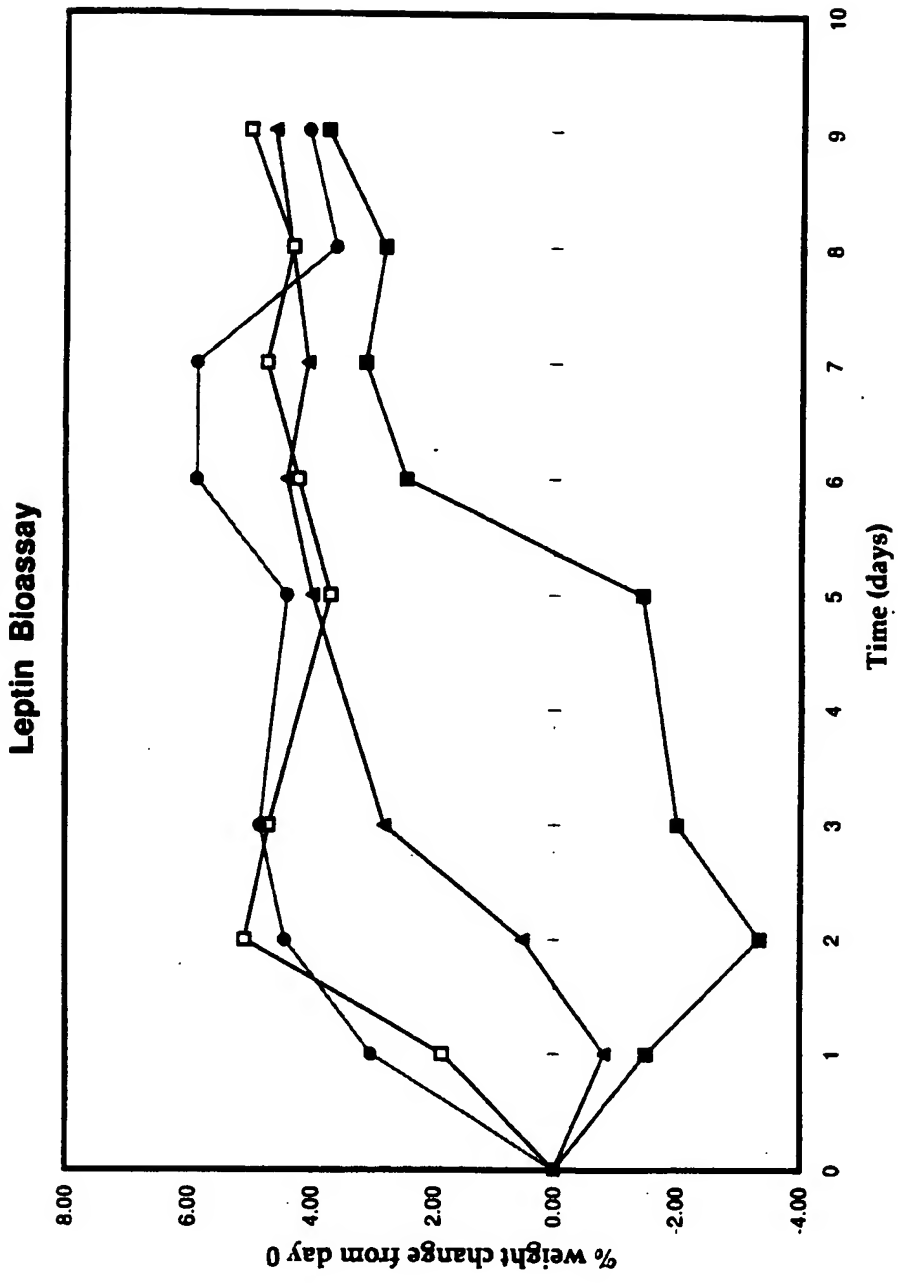

FIGURE 3